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Enviro-Chem Superfund Site Bio-Recirculation Pre-Design Investigation Work Plan

Revision 1

Prepared for

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1. INTRODUCTION

On behalf of the Trustee of the Environmental Conservation Chemical (ECC) Site Trust Fund, Geosyntec Consultants (Geosyntec), with the assistance of Ramboll US Consulting, Inc. (Ramboll), have completed this *Bio-Recirculation Investigation Work Plan* for the Enviro-Chem Superfund Site (or Site) located at 985 S. US Highway 421 in Zionsville, Indiana (**Figure 1**). This work plan outlines the field activities required to collect data to support development of a design for the full-scale bio-recirculation system.

1.1 Purpose

The purpose of this work plan is to evaluate the groundwater conditions in the Upper Sand and Gravel Unit in areas of the Site relevant to the bio-recirculation system design (i.e., upgradient edge of the Site where groundwater will be reinjected, downgradient edge of the Site where groundwater will be extracted, and from the approximate center of the Site where the former cooling water pond was historically located and a potential deeper source may exist). The specific design parameters of interest include characterizing the presence and concentrations of potential co-contaminants (if present) and understanding the geochemical conditions and natural microbiological community to evaluate potential for inhibition of microbial activity and/or the need for bioaugmentation. Select samples of groundwater and soils will be obtained from the Upper Till on Site to further understand the potential for co-contaminants that may leach into the underlying Upper Sand and Gravel Unit. In addition, an assessment of the aquifer hydraulics at the northern end of the Site near S-1 will be undertaken to better assess injection capacity.

The results from the proposed investigation will be used for the design of the active enhanced in situ bioremediation (EISB) recirculation remedy, which was selected for further development after discussion with United States Environmental Protection Agency (EPA) on the *Enviro-Chem Superfund Site Remedial Alternatives Analysis for the Area South of the Site* (RAA; Geosyntec, 2020a). Furthermore, it has been observed that the existing ECC Site groundwater treatment system fouls with carbonates and that there is the potential for iron fouling to occur as well. A review of the Site's geochemistry will also be performed to understand fouling mechanisms to allow for the effective design of the future injection wells, bio-fouling control, and ex situ treatment of a portion of the extracted groundwater.

1.2 Objectives

The specific objectives of the proposed work are to:

- Characterize the groundwater chemistry (i.e., co-contaminants, inhibitory conditions) and microbial community in the Upper Till and Upper Sand and Gravel Unit at select locations on the ECC Site;
- Evaluate the hydraulics in the area of the proposed injection wells by conducting step drawdown testing at S-1;
- Characterize the contaminant profile in the area of the former cooling water pond as shown in **Figure 2** and assess the extent to which residual contamination from the former cooling pond may have or may be continuing to migrate into the Upper Sand and Gravel Unit;
- Characterize the contaminant profile of source material that may be leaching into the Upper Sand and Gravel Unit through collection of a non-aqueous phase liquid (NAPL) sample for analytical testing if NAPL is recovered from the Upper Till well PT-11; and
- Review groundwater chemistry to confirm: (*i*) potential for inhibitory co-contaminants and/or geochemical conditions to be present on Site that may impact bioremediation performance; (*ii*) confirm the need for bioaugmentation to achieve degradation of the contaminant mass; and (*iii*) potential fouling mechanisms as seen in the former permeable reactive gate system (PRGS) site treatment system, which fouls with carbonates (i.e., water hardness).

1.3 Organization

This Work Plan is organized as follows:

- Section 2 presents a summary of the Site background;
- Section 3 describes the proposed investigation and sampling;
- Section 4 presents the project schedule; and
- Section 5 contains a list of all references.

2. BACKGROUND

2.1 Geology

The Site is underlain by five stratigraphic units, which are present in the following descending order (from shallowest to deepest):

(1) Superficial Fill of gravel and sand with well-graded and non-native sand in select areas of the site, including the off-Site area;

(2) Upper Till composed predominantly of clayey silt and silty clay with occasional lenses of sand and gravel;

(3) Upper Sand and Gravel Unit, containing fine to coarse sand and gravel with some silt lenses;

(4) Lower Till, an aquitard composed predominantly of clayey silt and silty clay; and

(5) Lower Sand and Gravel Unit containing dense sand lenses and finer-grained glacial tills.

A mixed glacio-fluvial/colluvial depositional environment is inferred for the Upper Sand and Gravel unit, which likely formed as post-glacial deposits from meltwater outwash. The remnants of a paleochannel, which is seen as a thickening of the Upper Sand and Gravel Unit, has been observed at the ECC Site.

This pre-design investigation focuses primarily on the Upper Sand and Gravel stratigraphic unit, which is to be treated by the bio-recirculation system, with some limited sample collection from the Upper Till to better understand the potential for inhibitory co-contaminants.

2.2 Hydrogeology

Table 1 summarizes the relevant hydrogeological characteristics of the Upper Till and Upper Sand and Gravel unit on Site (CH2M Hill, 1986) and in the off-Site area to the south of the ECC Site (Geosyntec, 2017). The Upper Till is a water-bearing unit of relatively low hydraulic conductivity and low groundwater velocities. Limited groundwater flow through the Upper Till primarily occurs through thin sand and gravel lenses. Groundwater velocities within the Upper Till are low (few feet per year; ft/yr).

The Upper Sand and Gravel Unit is similarly a water-bearing unit but is semi-confined. The Upper Till is thicker on the northern end of the ECC Site and thins to the south, and the semi-confined nature of the Upper Sand and Gravel Unit contributes to upward hydraulic gradients, particularly in the southern end of the ECC Site near Trench 6. The upward gradients mitigate contaminant migration from the Upper Till into the underlying Upper Sand and Gravel Unit.

On-site, hydraulic conductivities are approximately two orders of magnitude higher than the Upper Till, and groundwater velocity is similarly at least two orders of magnitude greater in the Upper Sand and Gravel Unit than in the Upper Till (**Table 1**).

On-Site, groundwater in the Upper Sand and Gravel Unit generally flows southward with a slight southeast component of flow towards the southeast corner of the Site. At the southern Site boundary, the Thin Barrier Curtain Wall (TBCW) extends approximately halfway into the Upper Sand and Gravel Unit over much of the Site boundary, which constricts (but does not prevent) groundwater flow to the south under the TBCW.

The flow restriction created by the TBCW results in a steeper hydraulic gradient and faster groundwater velocity under the TBCW and close to the Site as groundwater flows under the TBCW. As groundwater migrates away from the ECC Site, the gradient flattens out and groundwater velocities slow down again.

2.3 Nature and Extent of Contamination

The COCs for the ECC Site are the following volatile organic compounds (VOCs):

- 1,1,1-trichloroethane;
- 1,1,2-trichloroethane;
- 1,2-dichloroethene (cis and trans isomers);
- ethylbenzene;
- methylene chloride;
- tetrachloroethene;
- toluene;
- trichloroethene; and
- vinyl chloride.

As reported in the 1986 Remedial Investigation Report (RIR; CH2M Hill, 1986). additional compounds that were detected in groundwater prior to remedial actions being undertaken (including the EPA removal actions) included the following:

- 1,1-dichloroethene;
- Chloroethane;
- benzene;
- chloroform;
- Freon 11 (also known as trichlorofluoromethane); and
- phenol.

Some minor contaminant mass was discovered in three on-Site wells screened in the underlying Upper Sand and Gravel Unit in wells downgradient of the former cooling pond during 1983/84 investigations (CH2M Hill, 1986). Maximum concentrations detected included 98 µg/L chloroethane, 9 µg/L 1,1-dichloroethene, 13 µg/L trans-1,2-dichloroethene, 4 µg/L ethylbenzene, 64 µg/L methylene chloride[2], and 21 µg/L trichloroethene. There is uncertainty as to whether contamination of this Unit may have occurred via migration downwards through the Upper Till or through migration of contaminated water from the former cooling water pond (**Figure 2**), which intersected the Upper Sand and Gravel Unit, before its removal and backfilling. The extent of contamination within the Upper Sand and Gravel Unit was not well defined after the EPA removal actions in 1983 and 1984.

3. SCOPE OF WORK

The investigation activities presented in this work plan include sampling of groundwater monitoring wells, step-injection drawdown tests, characterization of the soil and groundwater in the area of the former cooling water pond, and reviewing the Site geochemistry to evaluate potential for microbial inhibition and/or fouling of the proposed recirculation wells and treatment system. The field investigation activities will be performed by Ramboll with support and reporting from Geosyntec. The subsequent subsections provide a description of the scope of work recommended by Geosyntec and Ramboll.

3.1 Monitoring Well Sampling

Groundwater sampling will be conducted at 10 Upper Sand and Gravel Unit monitoring wells (S-1, PS-1, PS-2, PS-3, PS-4, S-4B, S-9, S-7, S-11, and S-13) and three Upper Till wells (PT-6, PT-11, and T-13). Prior to groundwater sampling, a synoptic water level survey will be conducted at all wells. If a well has not been sampled in the previous two years it may first be re-developed to ensure that a representative sample can be collected. Prior to redevelopment, the well will be gauged with an oil-water interface probe to assess the presence of NAPL. If NAPL is detected, a sample of the NAPL will be collected for analysis prior to well development.

Groundwater will be purged using a QED Bladder pump or equivalent and clean, dedicated low-density polyethylene (LDPE) tubing for each well. Field parameters, including temperature, pH, specific conductance, oxidation-reduction potential (ORP), and dissolved oxygen (DO), will be recorded during purging. Samples will be collected using low-flow purging and sampling methods, in accordance with EPA-approved low-

[2] The presence of this compound may have been a result of lab contamination; however, the data were not B qualified in the report.

flow sampling methods, the Quality Assurance Project Plan (QAPP; EPA, 2005) and subsequent QAPP Amendments (Ramboll, 2016).

Groundwater samples will be collected for the following analyses (**Table 2**):

- Major cations (sodium, potassium, calcium, magnesium) and anions (chloride, nitrate, sulfate);
- Sulfide and nitrite;
- Dissolved and total metals;
- Alkalinity (total, carbonate and bicarbonate alkalinity);
- Total organic carbon;
- Volatile fatty acids;
- VOCs and SVOCs – full suite including Freon 11;
- Dissolved hydrocarbon gases; and
- Microbial assays for *Dehalococcoides*, *Dehalobacter*, *Dehalogenimonas*, vcrA (only from monitoring wells S-1, S-4B and S-13)

Microbial assays for *Dehalococcoides*, *Dehalobacter*, *Dehalogenimonas*, vcrA will be analyzed by SiREM of Knoxville, TN. **Appendix A** includes the procedures for groundwater sample collection and shipping instruction for analysis of microbial assays. One field duplicate for every ten samples will be collected for quality assurance and quality control (QA/QC) for VOCs, SVOCs, and Freon 11. In addition, a trip blank will be analyzed to establish no contamination has occurred during samples collection and transport of samples from the Site to the laboratory.

If NAPL is present in Upper Till well PT-11, which has been observed in this well in the past, then a sample of the NAPL will be collected for analysis of VOCs and SVOCs.

3.2 Hydraulic Testing

Slug and/or bail tests will be conducted at monitoring well S-1 to assess the hydraulics of the Upper Sand and Gravel Unit in the northern end of the Site and just north of the Site as consideration of potential injection points. The slug testing will consist of a minimum of three tests to confirm readings and use of a pressure transducer to get accurate water level readings. Following the slug testing, injection testing will be conducted at S-1 to assess operating parameters for full-scale injections. The injection testing will be short term and conducted using an estimate of 1,500 gallon of potable water. The need for a Class V injection permit will be evaluated but it is not expected to be required for this short during potable water injection test.

The injection test at well S-1 will be completed with potable water over a period of up to 4 hours. A pressure transducer will be installed in S-1 to monitor pressure changes during injection. Injection wellhead pressures will also be monitored. Water levels in the injection well will be monitored to gauge the hydraulic effects of the injection.

The injection and monitoring process is as follows:

- The top of the injection well will be sealed to prevent overflow;
- Injection will begin at a low rate (0.3 gallon per minute [gpm]) without pressurizing the injection well;
- The rate will then be incrementally increased in steps (target 3 gpm steps, with contingency to adjust these as needed to obtain a minimum of 3 steps within a 4-hour period), until such time as the pressure in the injection well indicates that the water elevation is above the top of casing (total pressure <20 pounds per square inch [psi]); and
- Each step-wise change in injection rate will only occur after water levels have stabilized (or nearly stabilized) in the injection well.
- Monitoring will be documented throughout the injection test, including measurement of injection flow rates, injection wellhead pressures, and automatic data collection using a pressure transducer.

3.3 Former Cooling Water Pond Area Characterization

Prior to the start of drilling activities, underground utilities and/or installations will be marked. Soil cores will be collected from two locations along the approximate centerline of the footprint of the former cooling water pond area (**Figure 2**). Prior to completion of the sampling activities, the RCRA cap material will be excavated using a low ground pressure (<11 psi), tracked excavator to expose the liner and the liner will be cut to allow access to the subsurface. Care will be taken in accessing the sampling locations to minimize any further damage to the RCRA cap.

A low ground pressure (<11 psi), tracked geoprobe will be used for subsurface investigation through the RCRA cap. Soil cores will be collected through the entire vertical profile through the Upper Till Unit and down through the Upper Sand and Gravel Unit to provide a depth discrete profile of contaminant concentrations through the target treatment depth. Soil cores will be screened in the field with a photoionization detector (PID) for the presence of VOCs. One soil sample will be collected from each 5-ft soil core from the portion of the cores with the greatest PID response. These soil samples will be retained for laboratory analysis. Soil samples will be collected using Terra Core® samplers and 4-ounce glass jars and stored on ice for transport to the analytical laboratory under chain of custody procedures. Soil samples will be submitted for analysis of VOCs and Freon 11 by EPA method 8260D and SVOCs by EPA method 8270E.

If a soil core exhibits elevated PID readings (> 500 ppm) at any depth or there is visual observation of DNAPL in the soil (oily phase on gloves or core liners, separate phase observed in soil pore spaces), then the borehole will be abandoned at that depth and the borehole backfilled with hydrated bentonite or grout to surface. A borehole will instead be advanced adjacent to the abandoned borehole by installing telescoping casing to the depth of the observed DNAPL to isolate that depth interval and the coring will then proceed to deeper intervals following the same procedures outlined above once the telescoping casing has been set. The exact location of the proposed soil core may be adjusted slightly based on access, subsurface or overhead obstructions and restrictions (e.g. above or below ground utilities). Additional excavation of the cap to expose more area for boring may be necessary if DNAPL is observed.

Up to 6 groundwater grab samples, one from the Till Unit and the remainder in the Sand and Gravel Unit, will also be collected from beneath the former cooling water pond immediately adjacent to the soil core using an SP16 Geoprobe© groundwater sampler (or equivalent) direct push sampler that allows for grouting from the bottom up in the borehole after sample collection. The groundwater samples will be analyzed for the same suite of analytes as the monitoring well sampling (**Table 2**).

After sampling activities are completed, the liner will be re-sealed, the excavated cap material replaced and any further damage to the cap due to sampling activities will be repaired.

3.4 Evaluation of Fouling Potential

Injection and extraction wells are commonly prone to fouling due to iron precipitation, excessive biomass growth, and/or carbonate precipitation (due to hardness), and may need to be cleaned periodically and/or controlled through biofouling control measures (such as daily pulsed injections of a biocide agent such as chlorine dioxide). Fouling of wells, piping and treatment equipment can over time reduce the ability to effectively capture the plume migrating beneath the southern Site boundary and/or the ability to reinject groundwater and amendments upgradient of the Site.

Iron precipitation often occurs in aerobic environments as the groundwater geochemistry is modified to anaerobic and reducing conditions with the injection of carbon substrate. Iron precipitation can be controlled by removing oxygen from the groundwater prior to reinjection and/or limiting the introduction of oxygen to the reinjected groundwater.

Carbonate precipitation is dependent on the degree of calcite saturation in groundwater (i.e., the hardness of the groundwater) and the partial pressure of carbon dioxide in the aquifer versus the surface. Often pH changes can help to dissolve precipitated carbonate and/or keep it in solution.

Biofouling is often exacerbated by the introduction of oxygen into the recirculated groundwater stream. Aerobic microorganisms grow at a much higher rate than anaerobic microorganisms that are typically responsible for dechlorination of chlorinated solvents. To mitigate biofouling, again avoiding the introduction of oxygen into the recirculated groundwater stream and/or stripping oxygen from the extracted groundwater prior to reinjection is needed. Daily pulsed injections of a biocide agent, such as chlorine dioxide, can also be very helpful in mitigating the impact of biofouling on groundwater recirculation equipment and wells. Occasional cleaning of well screens and piping may also be necessary using a substance such as glycolic acid, which breaks down biofilm at higher concentrations and is biodegradable at lower concentrations.

In order to reduce fouling in the wells that will be used for biorecirculation and the pump-and-treat system, a geochemist will review all groundwater chemistry data collected and perform geochemical modeling to determine the potential for fouling and the fouling mechanisms. This information will be used to assess appropriate engineering mitigation measures to reduce and control the impact of fouling on the treatment system.

3.5 Waste Management

One composited soil sample will be collected for laboratory analysis of toxicity characteristic leaching procedure (TCLP) parameters for soil waste characterization purposes.

All investigation derived waste (IDW) during drilling and sampling will be containerized in clean drums supplied by the drilling contractor on the same day that it is generated, labelled, and temporarily stored in a designated area until waste pickup.

3.6 Reporting

A factual letter report will be issued to EPA upon completion of the pre-design investigation detailing the methods and results of the investigations.

4. SCHEDULE

An anticipated schedule to complete the field investigation activities discussed in this work plan is presented below:

Task	Timeframe
Approval to proceed received from the EPA.	Week 0
Field preparation including scheduling, subcontractor contracting and Site access.	6 weeks
Completion of monitoring well sampling, hydraulic testing, and soil coring and groundwater sampling in the former cooling water pond area.	3 weeks - weather dependent
Compilation of field data report completed by Ramboll.	4 weeks
Analysis and generation of a report by Geosyntec that summarizes the finding of the results.	5 weeks
Total Number of Weeks to Completion Following Approval to Proceed	**18 weeks**

5. REFERENCES

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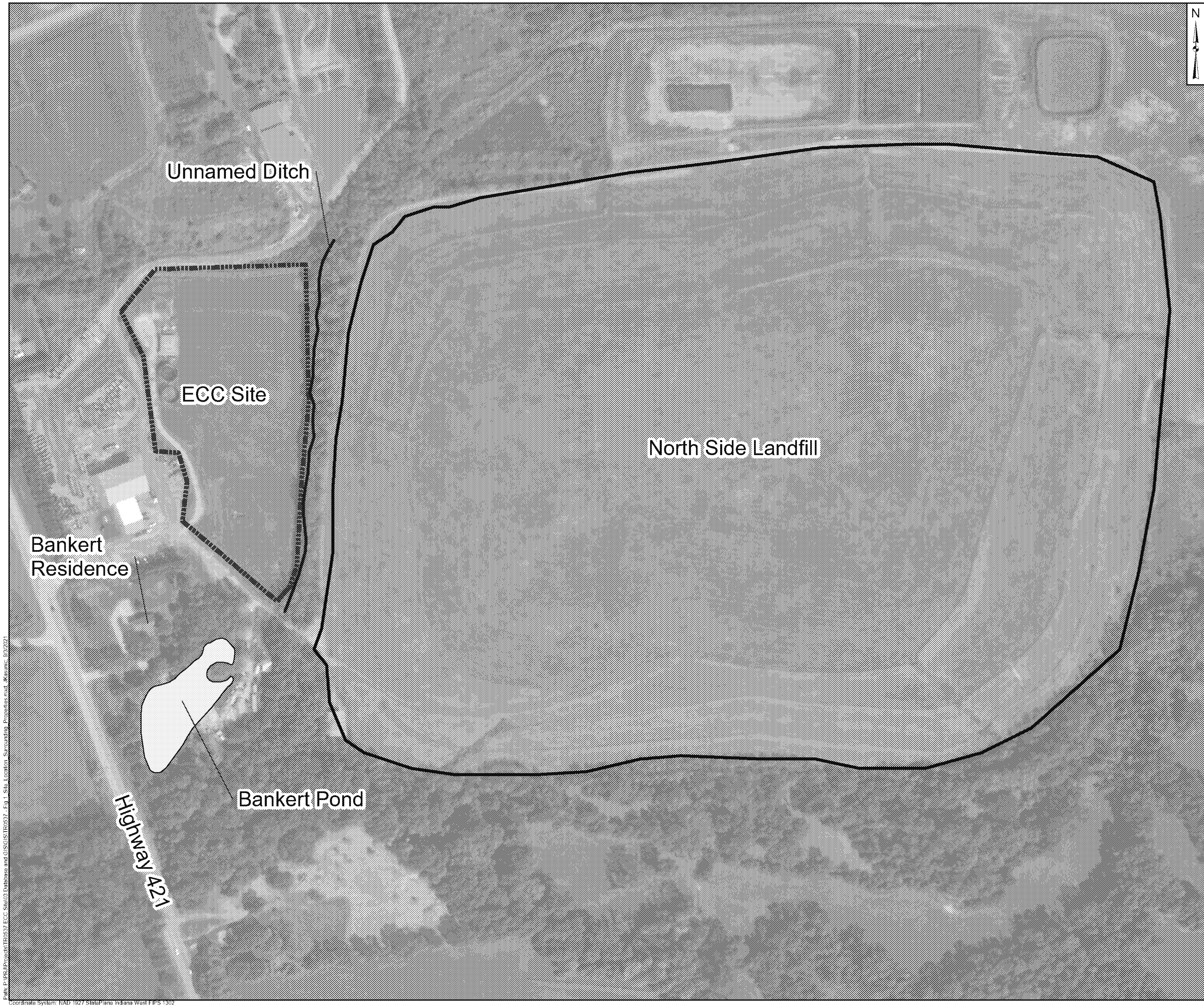
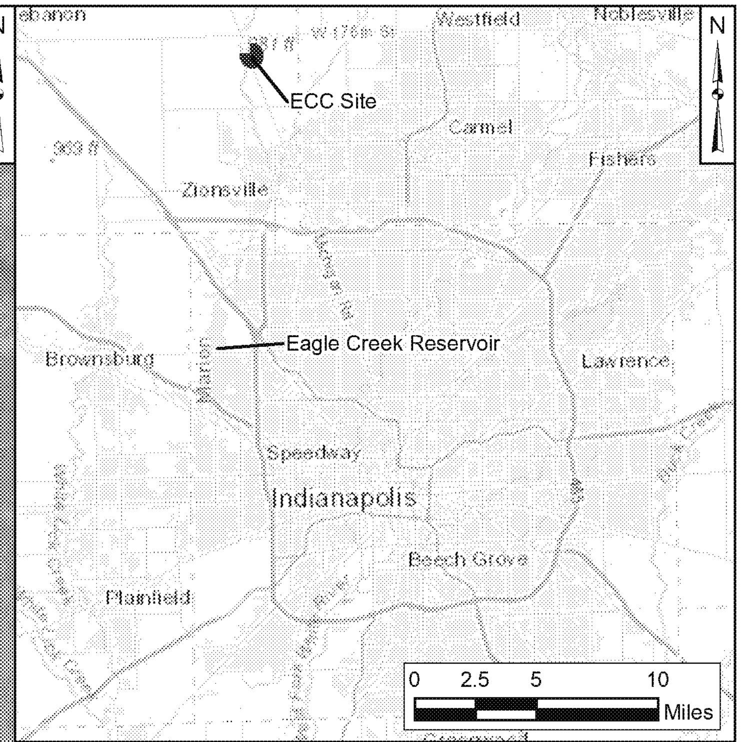
FIGURES





Legend

- Unnamed Ditch
- Site Boundary
- North Side Landfill
- Bankert Pond

Basemap Source: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

250 125 0 250 Feet

Site Location and Surrounding Properties

Enviro-Chem Superfund Site
985 South U.S. Highway 421
Zionsville, Indiana

Geosyntec consultants

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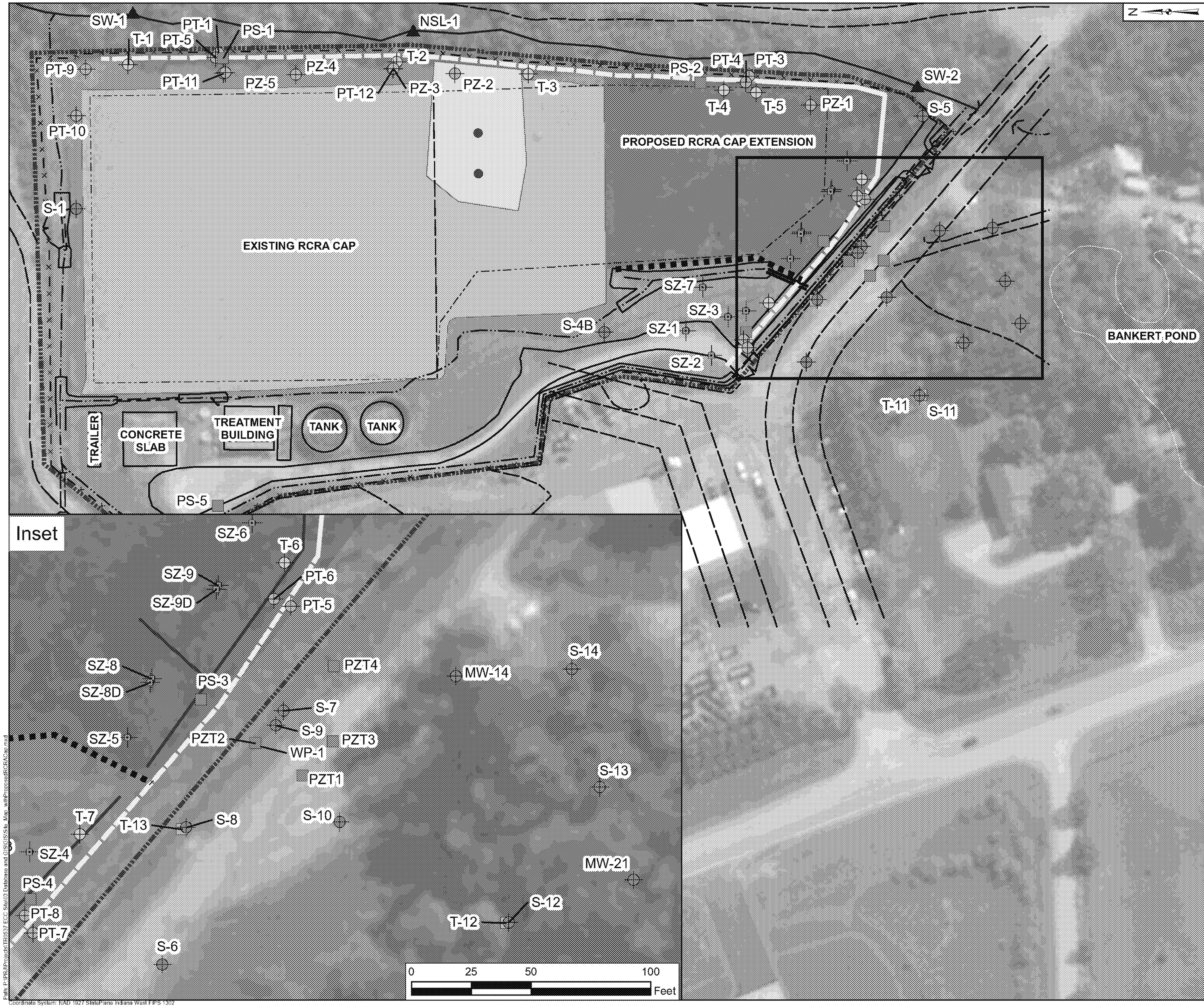
Figure 1

Coordinate System: NAD 1927 StatePlane Indiana West FIPS 1302



Legend

Groundwater Monitoring Locations

- Sand and Gravel Monitoring Well
- Sand and Gravel Piezometer
- Shallow (Till) Piezometer
- Shallow (Till) Well
- Support Zone Piezometer
- Surface Water Sampling Location
- Trench Dewatering Well
- Proposed Former Cooling Pond Sampling Location
- Property Extent
- Proposed Cut Off Wall
- Unnamed Ditch
- Proposed RCRA Cap Extension
- Existing RCRA Cap
- Approximate Location of Former Cooling Water Pond

Notes:

RCRA - Resource Conservation and Recovery Act
SVE - Soil Vapor Extraction
TBCW - Thin Barrier Curtain Wall

1. Basemap site features include the fenceline, concrete pad, RCRA cap and clay cover.
2. Solid green line indicates where the TBCW completely intersects the Upper Sand and Gravel unit; dashed line indicates that the TBCW does not completely intersect the Upper Sand and Gravel Unit.
3. Basemap Source: ESRI, DigitalGlobe, Geoeye, Earthstar, Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo.

100 50 0 100 Feet

Site Map

Enviro-Chem Superfund Site
985 South U.S. Highway 421
Zionsville, Indiana

Geosyntec consultants

Guelph | October 2021

Figure 2

TABLES

TABLE 1
HYDROGEOLOGICAL SUMMARY
ECC Superfund Site, Zionsville, Indiana

Lithological Unit	Area	Hydraulic Gradient (ft/ft)	Hydraulic Conductivity (ft/day)	Estimated Groundwater Velocity (ft/yr)
Upper Till	On-Site	0.02 to 0.05	0.03 to 0.3 (till) 1 (sand lenses)	1 to 2.6
Upper Sand and Gravel Unit	On-Site	0.028	5	100 to 1,000
	Off-Site	0.026	224	~3,650

Notes

The table summarizes the relavant hydrogeological characteristics of the Upper Till and Upper Sand and Gravel on Site as reported in CH2M Hill (1986).

Acronyms

ft/day - feet per day
ft/ft - feet per feet
ft/yr - feet per year
n/a - not available

TABLE 2
PROPOSED SAMPLING PROGRAM
ECC Superfund Site, Zionsville, Indiana

Location	Field Parameters	VOC and SVOC (including Freon 11)	Major Cations and Anions	Sulfide, Nitrite	Dissolved and Total Metals	Dissolved Hydrocarbon Gases	Alkalinity	Volatile Fatty Acids	Total Organic Carbon	Microbial assays for Dhc, Dhb, Dhg, vcrA
Upper Sand and Gravel Unit										
PS-1	x	x	x	x	x	x	x	x	x	
PS-2	x	x	x	x	x	x	x	x	x	
PS-3	x	x	x	x	x	x	x	x	x	
PS-4	x	x	x	x	x	x	x	x	x	
S-1	x	x	x	x	x	x	x	x	x	x
S-4B	x	x	x	x	x	x	x	x	x	x
S-7	x	x	x	x	x	x	x	x	x	
S-9	x	x	x	x	x	x	x	x	x	
S-11	x	x	x	x	x	x	x	x	x	
S-13	x	x	x	x	x	x	x	x	x	x
Former Cooling Water Pond Locations		x	x	x	x	x	x	x	x	
Upper Till										
PT-6	x	x	x	x	x	x	x	x	x	
PT-11	x	x	x	x	x	x	x	x	x	
T-13	x	x	x	x	x	x	x	x	x	
Duplicate		x								
Trip Blank		x								

Acronyms
Dhb - *Dehalobacter*
Dhc - *Dehalococcoides*
Dhg - *Dehalogenimonas*
SVOC - semi-volatile organic compounds
vcrA - vinyl chloride reductase
VOC - volatile organic compounds

APPENDIX A
Groundwater Sample Collection and Shipping for Microbial Analysis

GROUNDWATER SAMPLE COLLECTION AND SHIPPING FOR GENE-TRAC® ANALYSIS

This document provides sampling and shipping instructions for Gene-Trac® quantitative polymerase chain reaction (qPCR) (e.g., Gene-Trac® Dhc or FGA analysis) and Gene-Trac® next generation sequencing (NGS) tests performed on groundwater.

Sample Collection Methods: There are two groundwater sampling methods for Gene-Trac®:

- Method A: conventional groundwater sample collection; and
- Method B: field filtration (i.e., groundwater solids collected on a filter).

Both methods yield equivalent results; however, shipping charges for the field filters will be lower due to reduced size and weight of the samples retuned to the lab.

Ordering Sampling Supplies: SiREM is pleased to provide sampling supplies (containers or filters, coolers, ice packs upon request) free of charge. Note: Please provide 7 days advance notice for this service, otherwise a $50 shipping surcharge may apply. Please contact Ximena Druar 519-515-0838 / xdruar@siremlab.com or use our ***online sample kit order portal*** http://www.siremlab.com/sampling-supply-form to order sampling supplies.

Figure 1: Gene-Trac samples can be provided as either 1 L Wide mouth (HDPE) bottles (left) or Sterivex® filters (right). **Each filter is provided in an outer storage tube that contains the filter, a screw cap and a sample label**

Table 1: Sample Requirements for Gene-Trac® qPCR Testing and NGS Analysis

	Method A: Groundwater Sample	Method B: Field Filtration	Hold Time
Gene-Trac® qPCR Tests (e.g., Dhc/FGA)	One-1L Wide mouth Nalgene	One -Sterivex® filter with up to 1 L water passed through	7 days at 4°C
Gene-Trac® NGS	Two-1L Wide mouth Nalgene	Two-Sterivex® filters with up to 1 L water passed through	7 days at 4°C

Collecting Samples

For all Gene-Trac® (qPCR) tests, only one bottle or filter is required per sample. Please note that for next generation sequencing (NGS) analysis duplicate samples are required to provide sufficient biomass for analysis (SeeTable 1)

Groundwater Purging: Prior to groundwater sample collection, sampling points should be purged using industry-accepted well purging protocols to obtain representative groundwater. Note: turbidity in groundwater samples is not a concern.

Method A: Conventional Groundwater Samples in 1 Liter Bottles

Following purging, 1-liter (L) groundwater samples are collected in large mouth 1L high-density polyethylene (HDPE) bottles (e.g., Nalgene or equivalent) with minimal headspace. No preservatives are required; samples should be stored and shipped at 4ºC on blue or double bagged wet ice. The hold time is 7 days.

Method B: Field Filtration

1) Following ground water purging, remove Sterivex filter from storage container and insert luer-lock adapter (white barbed fitting) into pump effluent tubing (1/4”-5/16” inside diameter) and securely fasten using a hose clamp if required (Figure 1B).

2) Remove the white rubber nipple cover from the effluent end of the filter (do not discard cap-this will be used to seal after sampling).

3) Turn on pump and direct filter discharge into a graduated container (Figure 1B). Pass up to 1L of water through the filter. Note that the filter often clogs before a full 1L of sample is filtered. If this occurs, record the measured volume of water passed through the filter (in milliliters [mL]) on the label provided (Figure 1A) and the provided chain of custody. Shut off the pump.

4) Cap the effluent end of the filter (while full of water) with the small white nipple cap provided; decouple the tubing/luer-lock fitting from the influent end of the filter and seal the filter unit with the white screw-cap (Figure 1C). Place the sealed filter in the storage tube, label with the sample location, date and total volume of groundwater passed through the filter. The filter should be stored and shipped at 4ºC in the provided cooler (Figure 1D).

5) Remove the luer-lock fitting in the pump tubing and discard. Dispose of effluent groundwater in accordance with applicable site procedures.

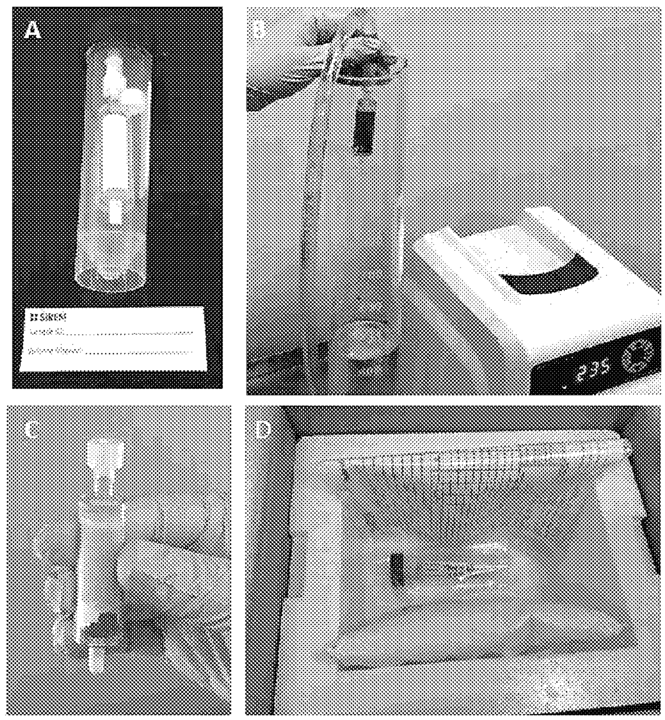


Figure 2: Use and Shipping of Field Filters

Labelling, Storage and Shipping

Sample Labelling and Handling: Samples should be clearly labeled (including sample ID and date) and individually sealed in re-sealable freezer bags provided and placed in a cooler with cool packs. If wet ice is used it must be double bagged. Sample hold time for 1L groundwater and filter samples is 7 days at 4°C.

Chain-of-Custody: Include the total volume passed through the filter for each sample (Method B only), the applicable purchase order number and quotation number where applicable. Please indicate which analysis is requested by noting the test method (refer to Attachment 1 for a list of Gene-Trac® analyses provided by SiREM). The completed chain-of-custody (Attachment 2) should be placed in a zip-lock bag inside the cooler with the samples.

Shipping: For samples originating in the USA ship samples by priority overnight courier to SiREM Knoxville, TN (address below). Samples should be given a nominal value of no more than $10.

Please note that SiREM is not open on Saturdays

The following shipping document is required:

Domestic Waybill (e.g., FedEx) see sample FedEx waybill (Attachment 3). Complete shipper specific information, other information should be completed as indicated.

Section 1: Fill in date, complete shipping address and include your FedEx account number
Section 2: Your internal reference number/project number (if required)
Section 3: To address is: (already completed)

SiREM Knoxville
180A Market Place Boulevard
Knoxville, TN 37922

Section 4a: Express package Service – mark FedEx priority overnight
Section 5: Other packaging
Section 7: Payment and by Sender
Section 8: Signature

Technical Inquiries:

- Ximena Druar 519-515-0838
  xdruar@siremlab.com

- Phil Dennis 519-515-0836
  pdennis@siremlab.com

Attachments: (1) Available Gene-Trac® Tests
(2) SiREM Chain-of-Custody
(3) Example FedEx Waybill

Attachment 1: Available Gene-Trac® Tests

Contaminant Class	Redox	Gene-Trac® Test Name	Target	Relevance
Chlorinated Ethenes	Anaerobic	Dhc	*Dehalococcoides*	Dechlorinates PCE, TCE, all DCE isomers, VC
		Dhb	*Dehalobacter*	Dechlorination of PCE &TCE to cDCE
		Dsm	*Desulfuromonas*	Dechlorination of PCE & TCE to cDCE
		Dsb	*Desulfitobacterium*	Partial dechlorination of PCE and TCE to cDCE
		Geo	*Geobacter*	Dechlorinates PCE to cDCE/biogeochemical degradation
		Dhg	*Dehalogenimonas*	Dechlorination of tDCE to VC and VC to ethene
		Chloroethene FGA	Vinyl Chloride Reductase (*vcrA*)	Dechlorination of cDCE & VC to ethene
			BAV1 Reductase (*bvcA*)	Dechlorination of cDCE and VC to ethene
			Trichloroethene Reductase (*tceA*)	Dechlorination of PCE and TCE to cDCE and VC
	Aerobic	Polaromonas	*Polaromonas*	Aerobic dechlorination of cDCE
		etn	*etnE*	Aerobic degradation of VC
Chlorinated Ethanes	Anaerobic	Dhb	*Dehalobacter*	Dechlorinates 1,1,1-TCA/1,2-DCA /1,1,2-TCA/ 1,1,2,2-TeCA
		Dhg	*Dehalogenimonas*	Dechlorinates 1,2- DCA, 1,1,2,2-TeCA , 1,1,2-TCA
		Dhc	*Dehalococcoides*	Dechlorinates 1,2-DCA to ethene
		Dsb	*Desulfitobacterium*	Dechlorinates 1,1,2-TCA &1,2-DCA
		cfrA/dcrA	Dichloroethane Dehalogenase (*dcrA*)	Dechlorinates 1,1,1-TCA & 1,1-DCA
	Aerobic	sMMO	Soluble Methane Monooxygenase	Co-metabolism of 1,1,1-TCA & 1,1-DCA by methanotrophs
		PMO	Propane Monooxygenase	Co-metabolism of chlorinated ethanes by propanotrophs
		dhlA	Haloalkane Dehalogenase (*dhlA*)	Aerobic dechlorination of 1,2-DCA
Chlorinated Methanes	Anaerobic	Dhb	*Dehalobacter*	Dechlorination of chloroform to DCM; DCM to acetate
		cfrA/dcrA	Chloroform Reductase (*cfrA*)	Converts chloroform to dichloromethane
	Aerobic	sMMO	Soluble Methane Monooxygenase	Co-metabolism of chloroform & dichloromethane
Chlorinated Propanes	Anaerobic	Dhg	*Dehalogenimonas*	Converts TCP to allyl chloride; DCP to propene
		Dhc	*Dehalococcoides*	Converts DCP to propene
		Dhb	*Dehalobacter*	Converts DCP to propene
		Dsb	*Desulfitobacterium*	Dechlorination of TCP & DCP
Chlorinated Benzenes	Anaerobic	Dhc	*Dehalococcoides*	Partial dechlorination of HCB/PCB
		Dhb	*Dehalobacter*	Reductive dechlorination of DCB, MCB
Chlorinated Phenols	Anaerobic	Dhc	*Dehalococcoides*	Dechlorination of 2,3-dichlorophenol, TCP and PCP
PCBs	Anaerobic	Dhc	*Dehalococcoides*	Dechlorinates select Arochlor 1260 congeners
		Dhb	*Dehalobacter*	Dechlorinates 2,3,4-trichorobiphenyl; 2,3,4,5-tetrachlorobiphenyl
		Dhg	*Dehalogenimonas*	Dechlorinates select Arochlor 1260 congeners
BTEX	Anaerobic	SRB	Sulfate reducing bacteria (*dsrA*)	Partners to ORM-2 in anaerobic benzene degradation
		ORM-2	*Deltaproteobacterium ORM-2*	Anaerobic benzene degrader (SO_4/CH_4 reducing conditions)
		Pepto-ben	Benzene degrading *Peptococcaceae*	Anaerobic benzene degrader under NO_3 reducing conditions
		abcA	Benzene Carboxylase (*abcA*)	Involved in benzene ring cleavage
Fuel Oxygenates	Aerobic	MTBE/TBA	*Methylibium petroleiphilum* PM1	MTBE/TBE degrading microorganism
			tert-butyl alcohol hydroxylase (*mdpJ*)	Active on TBA in aerobic MTBE degradation pathway
			HIBA mutase (*hcmA*)	Active on 2-HIBA in aerobic MTBE degradation pathway
1,4-Dioxane	Aerobic metabolism	1,4-dioxane	Dioxane monooxygenase (*dxmb*)	Energy yielding 1,4-dioxane degradation
		1,4-dioxane	Aldehyde Dehydrogenase	Energy yielding 1,4-dioxane degradation
	Aerobic Cometabolism	pMMO	Particulate Methane Monooxygenase	Co-oxidation of 1,4-dioxane in presence of methane
		sMMO	Soluble Methane Monooxygenase	Co-oxidation of 1,4-dioxane
		PMO	Propane Monooxygenase	Co-oxidation of 1,4-dioxane in presence of propane
Nitrogen	Anaerobic	Anammox	Major anammox genera	Anaerobic co-removal of ammonium and nitrite
Prokaryotic Groups	Variable	Universal	Bacteria	Quantifies *Bacteria*-measure of total biomass
		Arch	Archaea	Quantifies *Archaea* biomass
		SRB	Sulfate reducing bacteria (*dsrA*)	Anaerobic hydrocarbon oxidation/biogeochemical reduction/MIC
		NGS	*Bacteria/Archaea*	Comprehensive characterization of microbial communities

Attachment 2:

SiREM Chain-of-Custody

SiREM

Chain-of-Custody Form

siremlab.com

130 Stone Rd. W
Guelph, ON N1G 3Z2
(519) 822-2265

Lab #

*Project Name	*Project #
*Project Manager	*Company
*Email Address	
Address (Street)	

City	State/Province	Country

*Phone #

*Sampler's Signature	*Sampler's Printed Name

Analysis

Client Sample ID	Sampling Date	Sampling Time	Matrix	# of Containers	Gene-Trac DHC	Gene-Trac FGA	Gene-Trac DHB	Gene-Trac DHG	Volatile Fatty Acids	Dissolved hydrocarbon gases	Treatability Study					Other Information

Preservative Key

0. None
1. HCL
2. Other ______
3. Other ______
4. Other ______
5. Other ______
6. Other ______

Billing Information

P.O. #

*Bill To:

Turnaround Time Requested

Normal ☐

Rush ☐

For Lab Use Only

Cooler Condition:

Cooler Temperature:

Custody Seals: Yes ☐ No ☐

For Lab Use Only

Proposal #: ______

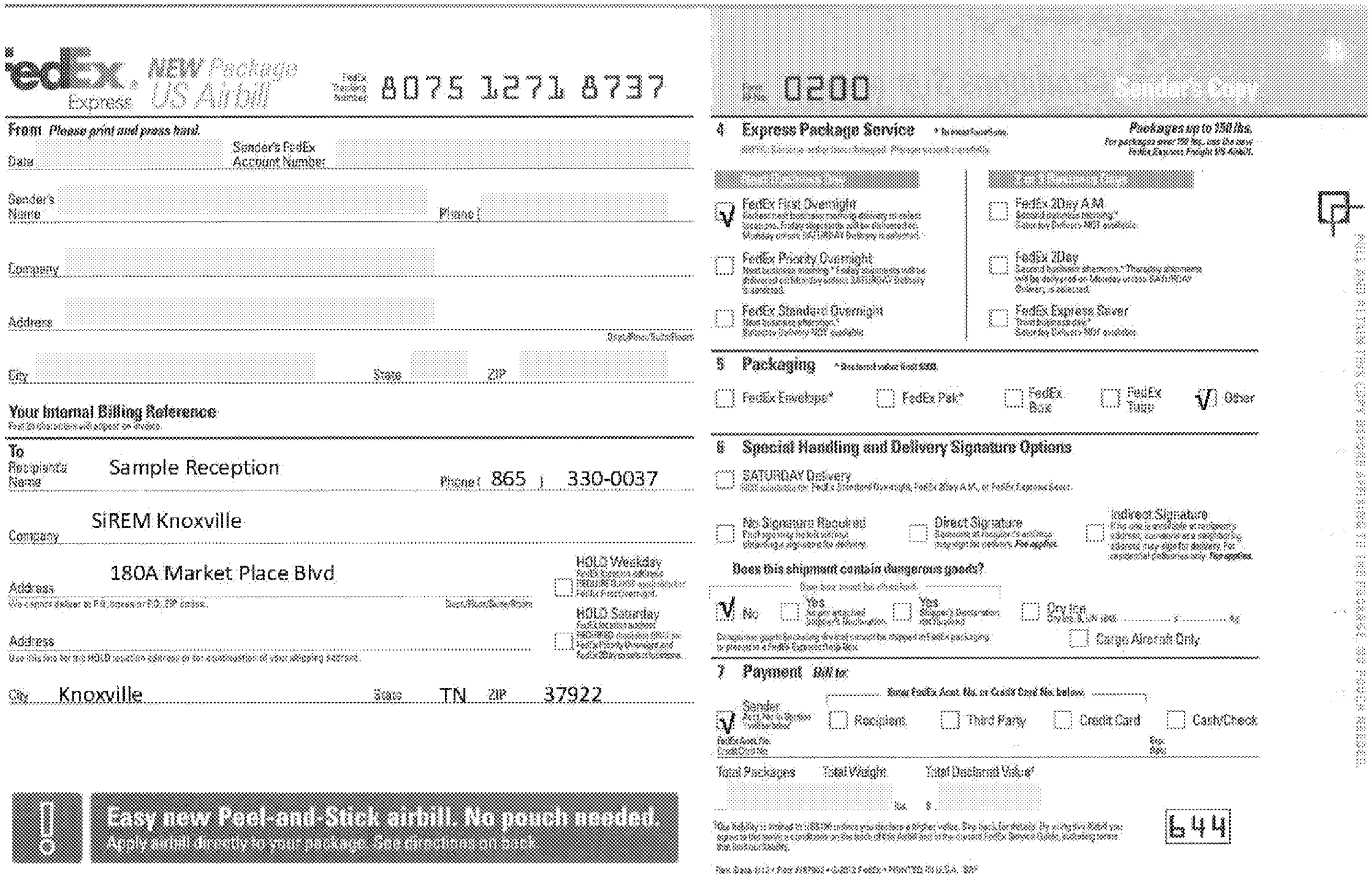
Relinquished By:	Received By:	Relinquished By:	Received By:	Relinquished By:	Received By:
Signature	Signature	Signature	Signature	Signature	Signature
Printed Name	Printed Name	Printed Name	Printed Name	Printed Name	Printed Name
Firm	Firm	Firm	Firm	Firm	Firm
Date/Time	Date/Time	Date/Time	Date/Time	Date/Time	Date/Time

Distribution: White - return to Originator; Yellow - Lab Copy; Pink - Retained by Client

** Mandatory Fields*

Attachment 3:

Example FedEx Waybill



FedEx Express NEW Package US Airbill
FedEx Tracking Number 8075 1271 8737
Form 0200
Sender's Copy

From *Please print and press hard.*
Date
Sender's FedEx Account Number
Sender's Name
Phone
Company
Address
City
State
ZIP

Your Internal Billing Reference

To
Recipient's Name: Sample Reception
Phone (865) 330-0037
Company: SiREM Knoxville
Address: 180A Market Place Blvd
HOLD Weekday
HOLD Saturday
Address
City: Knoxville
State: TN
ZIP: 37922

Easy new Peel-and-Stick airbill. No pouch needed.
Apply airbill directly to your package. See directions on back.

4 Express Package Service
Packages up to 150 lbs.
- [x] FedEx First Overnight
- [ ] FedEx Priority Overnight
- [ ] FedEx Standard Overnight
- [ ] FedEx 2Day A.M.
- [ ] FedEx 2Day
- [ ] FedEx Express Saver

5 Packaging
- [ ] FedEx Envelope*
- [ ] FedEx Pak*
- [ ] FedEx Box
- [ ] FedEx Tube
- [x] Other

6 Special Handling and Delivery Signature Options
- [ ] SATURDAY Delivery
- [ ] No Signature Required
- [ ] Direct Signature
- [ ] Indirect Signature

Does this shipment contain dangerous goods?
- [x] No
- [ ] Yes
- [ ] Yes
- [ ] Dry Ice
- [ ] Cargo Aircraft Only

7 Payment *Bill to:*
- [x] Sender
- [ ] Recipient
- [ ] Third Party
- [ ] Credit Card
- [ ] Cash/Check

Total Packages
Total Weight
Total Declared Value

644